domains (CBD) by a glycosylated linker peptides (see FIG. 1) (Koivula et al., (1996) *Protein Expression and Purification* 8:391-400). The catalytic domain hydrolyzes the mannan, the CBD type domains increase the concentration of the enzyme on the substrate, in this case hemicellulose, and the linker peptides provide flexibility.

Please replace the fifth full paragraph on page 16, beginning line 24, with the following paragraph:

As described more fully in the Examples below, ManA, a novel thermostable mannanase, has now been identified and characterized. The predicted amino acid sequence of ManA (SEQ ID NO:1) has an organization characteristic of a mannanase enzyme. ManA contains a GH5 catalytic domain (about amino acid 37 to 411)-linker domain-carbohydrate binding type III domain (about amino acid 455 to 608) organization, as well as a second carbohydrate binding type II domain (about amino acid 662 to 762). As discussed in more detail below, significant amino acid similarity of ManA to other mannanases identifies ManA as a mannanase.

**REMARKS**

The present paper is submitted as a complete response to the Notice mailed August 22, 2001. Applicants respectfully request that the present papers be made of record.

Should any additional issues need to be resolved, the Examiner is requested to telephone the undersigned to attempt to resolve those issues. If a further written action is required, Applicant requests that the prior final rejection be withdrawn for the reasons noted above.

Respectfully submitted,

Paul J. White, Reg. No. 30,436
Attorney for Applicants

Dated: October 22, 2001.

National Renewable Energy Laboratory
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UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Shi-You Ding, et al.. Docket No.: NREL 01-35

Serial No.: 09/917,378

Filed: July 28, 2001

Title: THERMAL TOLERANT MANNANASE FROM CELLULASE FROM ACIDOTHERMUS CELLULOLYTICUS

**SPECIFICATION AMENDMENTS -- MARKUP**

Please replace the first full paragraph on page 2, beginning line 2, with the following paragraph:

A particularly rich source of mannans is the hemicellulose content of softwood, and in particular, the waste material from softwood processing in paper manufacturing. One of the more important hemicelluloses in softwood is galactoglucomannan, which is composed of a backbone of β-(1,4)-linked D-mannopyranose and D-glucopyranose in a ratio of approximately 3:1, respectively (Sjostrom, E. (1992) Wood Chemistry, 2nd Ed., Academic Press: New York, NY, pp 63-70). Other sources of mannans include the endosperm of copra [capra] and ivory palm nuts, guar beans, coffee beans, and roots of konjak (*Amorphorphallus konjac*).

Please replace the third full paragraph on page 13, beginning line 21, with the following paragraph:

"Thermal tolerant" refers to the property of withstanding partial or complete inactivation by heat and can also be described as thermal resistance or thermal stability. Although some variation exists in the literature, the following definitions can be considered typical for the optimum temperature range of stability and activity for enzymes: psychrophilic (below freezing to 10C); mesophilic (10°C to 50°C); thermophilic (50°C to 75°C); and caldophilic (75°C to above boiling water temperature). The stability and catalytic activity of enzymes are linked characteristics, and the ways of measuring these properties vary considerably. For industrial enzymes, stability and activity are best measured under use conditions, often in the presence of substrate. Therefore, mannanases that must act on process streams of mannans must be able to withstand exposure up to thermophilic or

even caldophilic temperatures for digestion times in excess of several hours.

Please replace the first full paragraph on page 16, beginning line 2, with the following paragraph:

Mannanases are characterized by having a multiple domain unit within their overall structure;[,] a GH or catalytic domain is joined to a carbohydrate binding type II and III domains (CBD) by a glycosylated linker peptides (see FIG. 1) (Koivula et al., (1996) *Protein Expression and Purification* 8:391-400). The catalytic domain hydrolyzes[sis] the mannan, the CBD type domains increase[s] the concentration of the enzyme on the substrate, in this case hemicellulose, and the linker peptides provide[s] flexibility[ for both larger domains].

Please replace the fifth full paragraph on page 16, beginning line 24, with the following paragraph:

As described more fully in the Examples below, ManA, a novel thermostable mannanase, has now been identified and characterized. The predicted amino acid sequence of ManA (SEQ ID NO:1) has an organization characteristic of a mannanase enzyme. ManA contains a GH5 catalytic domain (about amino acid 37 to 411)-linker domain-carbohydrate binding type III domain (about amino acid 455 to 608) organization, as well as a second carbohydrate binding type II domain (about amino acid 662 to 762). As discussed in more detail below, significant amino acid similarity of ManA to other mannanases identifies ManA as a mannanase.